ORIGINAL RESEARCH

6,6′-Dimethoxygossypol: molecular structure, crystal polymorphism, and solvate formation

Carlos A. Zelaya · Edwin D. Stevens · Michael K. Dowd

Abstract 6,6′-Dimethoxygossypol (DMG) is a naturally occurring derivative of gossypol that is found in relatively high concentration in some *Gossypium barbadense* cotton varieties. Like gossypol, DMG forms an equimolar solvate with acetic acid, but it was not known if, like gossypol, the compound would also form clathrates with other molecules. To test for this, the compound was crystallized from different solvents. Four new structures of DMG were found that include two polymorphic and two solvated forms. The polymorphs include two monoclinic structures with $P2_1/c$ and $C2/c$ space groups (**P1** and **P2**, respectively). Packing of the DMG molecules **P1** is similar to packing of the gossypol molecules in the **P1** polymorphic form of gossypol. The DMG molecules in **P2** pack in a highly ordered arrangement that has not been previously observed among gossypol structures. DMG forms equimolar solvates with water (**S1**) and cyclohexanone (**S2**). Both structures are triclinic with $P\bar{1}$ space groups. The DMG molecules in **S2** assemble in a manner that is similar to the gossypol molecules of gossypol–cyclohexanone (1:1), and the DMG molecules in **S1** pack in a manner that is similar to the DMG molecules in DMG–acetic acid (1:1) as well as the gossypol molecules in gossypol–acetic acid (1:1). Although DMG is not as versatile a host compound as gossypol, it still forms solvates under many crystallization conditions. Consequently, some care is needed to be sure that one understands exactly which form is recovered when the compound is isolated.

Introduction

Gossypol is a polyphenolic terpene that is found in many tissues of the cotton plant (*Gossypium* sp.). The presence of the compound in cottonseed limits the use of cottonseed products as a protein ingredient in ruminant feeds [1]; however, the compound also has an interesting array of potentially useful bioactivity, including anticancer and antiviral effects [2–5]. Gossypol also exhibits a number of unusual physical properties. Among these properties is its tendency to form different solid-state forms depending on the crystallization conditions used during its isolation [6]. Many of these structures are clathrates (or solvates) that form when the compound is crystallized from different solvents or in the presence of small guest molecules. Gossypol has also been found to form multiple solid-state structures with same guest molecule, e.g., solvates formed with dichloromethane [7] and acetone [8, 9]. Non-solvated and de-solvated polymorphs of the compound are also known [6, 10].

6,6′-Dimethoxygossypol (DMG) (Fig. 1) is a related terpene that can be synthesized from gossypol by full methylation to form hexamethoxygossypol followed by acid hydrolysis to remove four of the methyl groups [11]. The compound also exists as a natural product in the roots and seeds of some cotton species and varieties [12]. During its original synthesis, the compound was found to form an equimolar solvate with acetic acid, and it was reported that

C. A. Zelaya · E. D. Stevens
Department of Chemistry, University of New Orleans,
New Orleans, LA 70148, USA

M. K. Dowd (✉)
Southern Regional Research Center, ARS, USDA,
1100 Robert E. Lee Blvd., New Orleans, LA 70124, USA
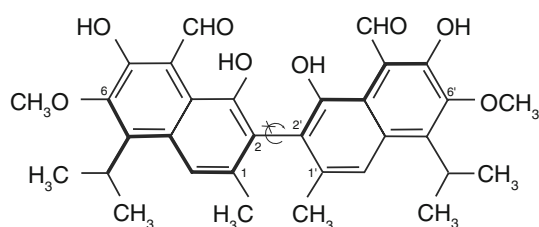e-mail: michael.dowd@ars.usda.gov

Fig. 1 Structure of 6,6′-dimethoxygossypol

the acetic acid could be removed by re-crystallization from benzene and hexane [11]. Our group has recently isolated DMG from the root bark of *Gossypium barbadense* St. Vincent Superfine cotton [13, 14]. During this isolation, the compound was recovered as an equimolar solvate with acetic acid [13]. A single crystal of this solid-state form was subsequently produced, and a diffraction study [15] showed that its structure is similar to the structure of the well-known clathrate formed between gossypol and acetic acid, i.e., gossypol–acetic acid (1:1) [16]. Because DMG and gossypol both form acetic acid solvates and because of the prior mention of a non-solvated form of DMG, it was decided to determine if DMG, like gossypol, would form different solid-state structures under different crystallization conditions. From this study, four new solid-state forms of DMG were found, and their structures were determined by X-ray diffraction.

Methods

DMG–acetic acid (1:1) (4–5 mg) was dissolved in acetone, diethyl ether, cyclohexanone, pentan-2-one, and chloroform (typically ∼200 μL). Petroleum ether (PE) (1–1.5 mL) was then added to each solution to produce supersaturated conditions. Samples were then allowed to stand at room temperature (20 ± 2 °C) for extended periods of time. The two DMG polymorphs (**P1** and **P2**) were produced from acetone and diethyl ether (and other conditions). We expect that the polymorph obtained is sensitive to the temperature during crystallization, although this was not explicitly studied. Surprising, DMG–water (1:1) (**S1**) was obtained from the chloroform/PE and pentan-2-one/PE solutions. From chloroform, the crystallization required several months. Presumably, this extended period was needed to allow for the diffusion of water vapor into the vial. The pentan-2-one sample used was known to have a small amount of contaminating water (∼1%). We have had some difficulty in reliably growing this crystal form, which we believe is due to its formation requiring a very narrow range of conditions (temperature and water concentration). Our repeated attempts to regenerate this structure from water-saturated chloroform or pentan-2-one generally resulted in

the formation of one of the non-solvated polymorphs. The DMG–cyclohexanone crystal (**S2**) was obtained from the cyclohexanone/PE solution.

Diffraction data were collected with a Bruker diffractometer fitted with a graphite monochromator and a SMART 1K CCD detector (Table 1). Except for **S1**, diffraction data were collected at sub-ambient temperatures (180–200 K). For **S1**, data were collected at room temperature because of frosting problems with the liquid nitrogen flow cryostream. At least two full sets of ϕ and ω scans were collected for each structure. Bruker SMART and SAINT software was used to collect and integrate the peak intensities [17], and SHELX NT (version 5.1) was used for the structure solution and refinement [18]. The structures were solved by direct methods and were refined by least squares fitting of F^2 over all unique reflections. All non-hydrogen atoms were modeled anisotropically. In general, hydrogen atoms were found in difference maps and were refined isotropically; however, several methyl and methylene hydrogen atoms, especially those associated with disordered groups, were placed at calculated positions to improve their geometries. The cyclohexanone molecule in **S2** was also found to be disordered. As the solid-state conformation of cyclohexanone is a "chair" form [19], this molecule was modeled in two essentially "inverted" chair conformations, and the methylene hydrogen atoms were placed at calculated positions to improve their geometries. DELU and SIMU restraints were also used for some structures to improve their thermal motion parameters [18].

Atom labels were chosen to be as consistent as possible with previous gossypol studies [6]. The DMG polymorph with the C2/c spacegroup (**P2**), however, has an asymmetric unit of ½ of the DMG molecule, and the atom numbering of this structure necessarily differs from that of the other structures (Fig. 2). For the three structures lacking this internal symmetry, the carbon atoms of the two naphthalene rings are labeled 1–10 and 11–20; the five carbon atoms surrounding the first naphthalene ring are numbered 21–25; and the five carbon atoms surrounding the second naphthalene ring are numbered 26–30. Oxygen atoms decorating the first naphthalene ring are labeled O1 through O4, and oxygen atoms decorating the second naphthalene ring are labeled O5 through O8. The two methoxy methyl groups are labeled C31 and C32. The minor disordered parts of the isopropyl groups are labeled with the numbers for the major part but increased by ten units, e.g., C23 for the major part and C33 for the minor part. Labeling of solvent molecules starts with C40 and O9, with the minor part of the cyclohexanone molecule starting at C50. For the half-molecule within the asymmetric unit of **P2**, the naphthalene ring atoms are numbered 1 through 10; the carbon atoms decorating the naphthalene ring are numbered C21 through C25; and the oxygen atoms are labeled O1 through O4, as they
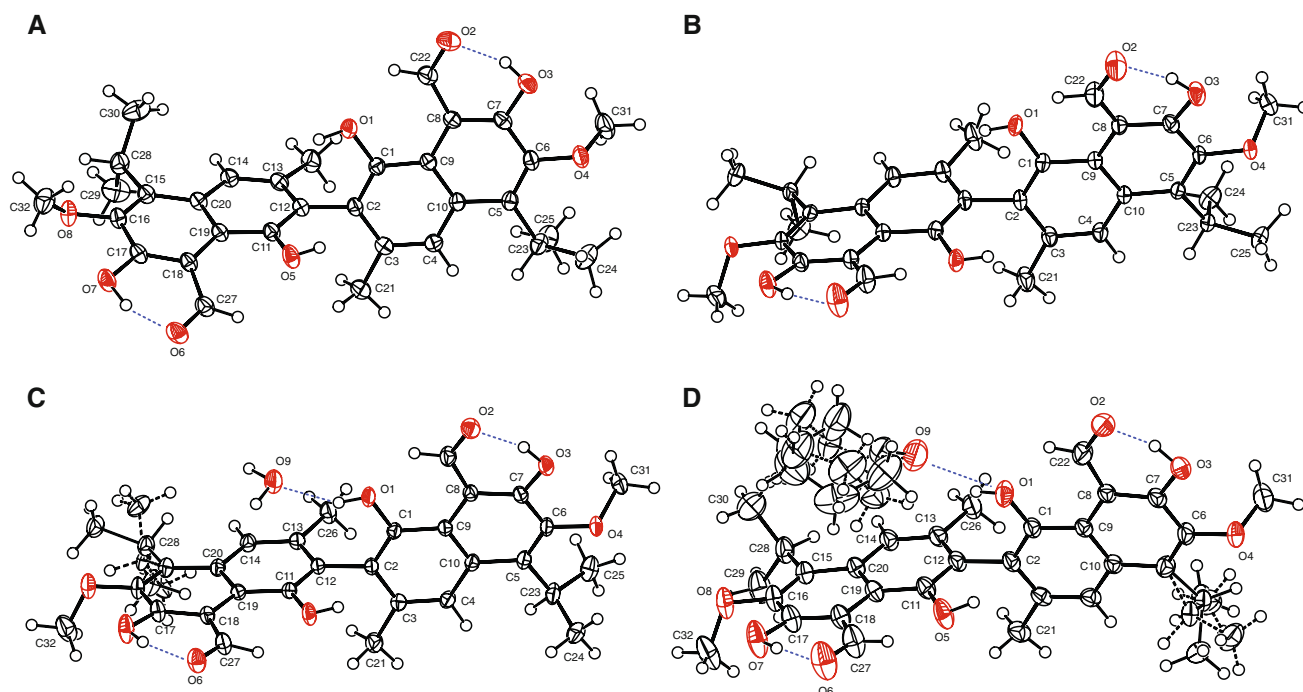
Fig. 2 ORTEP figures for 6,6′-dimethoxygossypol (DMG) crystal structures. **a** DMG (**P1**), **b** DMG (**P2**), **c** DMG–water (1:1) (**S1**), **d** DMG–cyclohexanone (1:1) (**S2**). Most atoms labeling is shown. For disordered parts, atom labeling is given in Fig. 3

are in the gossypol-1,4-dioxane (1:3) solvate, which has similar internal symmetry [20]. The methoxy carbon atom is labeled C31. The added symmetry of this structure can cause confusion in the comparison of hydrogen bonding patterns and molecular packing between the two types of structures. In general, discussion of the structure will refer to the labeling of the three less symmetrical DMG structures with appropriate differences noted for **P2**. Figures were generated with PLATON [21].

Results

Molecular conformation

DMG molecules in all four crystal forms are found in the aldehyde tautomeric form (Fig. 2), which is the same tautomeric form observed in all known gossypol crystal structures [6]. Intramolecular hydrogen bonds are observed in DMG between the O3–H hydroxyl hydrogen atom and the O2 carbonyl oxygen atom and between the O7–H hydroxyl hydrogen atom and the O6 carbonyl oxygen atom, as they are in all gossypol structures [6]. There are relatively few instances of the O3–H and O7–H hydroxyl hydrogen atoms of gossypol or DMG also donating into intermolecular hydrogen bonds, which suggests that the intramolecular interactions are very strong. In each DMG structure, the methoxy groups are rotated away from the plane of the

naphthalene rings by ∼100–120° (Table 2), similar to the orientation of these groups in DMG–acetic acid (1:1) [15]. In gossypol crystals, the equivalent hydroxyl hydrogen atoms are usually oriented within the extended naphthalene planes because of the formation of weak intramolecular hydrogen bonds between these hydroxyl groups and the adjacent *ortho*-positioned O4 and O8 hydroxyl groups [6] (Table 3).

The bridged naphthalene rings of each molecule are oriented approximately perpendicular to each other with angles between best-fit naphthalene ring planes (relative to the same sides of the rings) ranging from 84.3° to 104.9° (Table 2). Atom deviations from these planes are generally small; however, the root-mean-squared average deviation for the atoms of at least one the naphthalene rings of the DMG polymorphs is generally larger than for the DMG solvated forms (Table 2). A similar difference has been noted among gossypol polymorphs and solvates [6].

Some differences are found in the orientation of the isopropyl groups of the four DMG crystal forms, which has also been observed within gossypol crystal forms [6]. Most of the isopropyl groups of the DMG molecules are oriented with the methyl groups directed outward and away from the center of the molecule, which is also the most common orientation for the isopropyl methyl groups in gossypol crystal forms. This orientation, however, results in a short contact distance between the isopropyl ternary hydrogen atom and the naphthalene hydrogen atom at the 4-position, i.e., between the H4 and H23 atoms and between the H14

Table 1 Selected crystal, diffraction and refinement data for 6,6′-dimethoxygossypol (DMG) crystal forms

| Structure | DMG | DMG | DMG–water (1:1) | DMG–cyclohexanone (1:1) |
	P1	**P2**	**S1**	**S2**
Crystal data				
CCDC number	743862	743860	743859	743861
Empirical formula	$C_{32}H_{34}O_8$	$C_{32}H_{34}O_8$	$C_{32}H_{34}O_8 \cdot H_2O$	$C_{32}H_{34}O_8 \cdot C_6H_{12}O$
Formula wt., Z	546.59, 4	546.59, 4	564.61, 2	644.73, 2
Color, habit	Orange, plate	Orange, chip from rod	Orange, thick rod	Orange, plate
Size (mm)	$0.25 \times 0.5 \times 0.5$	$0.3 \times 0.3 \times 0.5$	$0.2 \times 0.4 \times 0.6$	$0.2 \times 0.6 \times 0.6$
Crystal system	Monoclinic	Monoclinic	Triclinic	Triclinic
Space group	$P2_1/c$	$C2/c$	$P\bar{1}$	$P\bar{1}$
Unit cell dimensions (Å and °)				
a	13.1324(7)	10.1266(4)	7.3473(8)	10.6468(4)
b	21.535(1)	15.3231(5)	13.192(2)	11.0379(6)
c	9.5208(5)	17.7160(6)	14.474(2)	15.5217(8)
α	90	90	82.485(2)	74.719(1)
β	98.535(1)	96.834(1)	82.896(2)	73.946(1)
γ	90	90	85.732(3)	79.382(1)
Cell volume (Å^3)	2662.7(2)	2729.5(2)	1377.7(3)	1678.7(2)
Cal. density (g/cm^3)	1.363	1.330	1.361	1.275
F(000)	1160	1160	600	688
μ (mm^{-1})	0.098	0.095	0.099	0.090
Data collection				
Temperature (K)	200(2)	180(2)	293(2)	200(2)
2θ range (°)	4.72–55.00	4.64–52.88	4.48–50.00	5.22–56.68
Index range	$-17 \leq h \leq 17$	$-12 \leq h \leq 12$	$-8 \leq h \leq 8$	$-14 \leq h \leq 14$
	$-27 \leq k \leq 27$	$-19 \leq k \leq 19$	$-15 \leq k \leq 15$	$-14 \leq k \leq 14$
	$-12 \leq l \leq 12$	$-22 \leq l \leq 22$	$-17 \leq l \leq 17$	$-20 \leq l \leq 20$
Reflections				
Total	35540	19139	13257	19867
Unique	6116	2828	4838	8346
Observed ($>2\sigma(I)$)	5241	2495	4284	6093
R_{int}	0.0320	0.0204	0.0261	0.0226
R_{σ}	0.0183	0.0138	0.0292	0.0287
Completeness to 2θ	99.9	100	99.5	99.4
Extinction coefficient	0.005(1)	0	0	0
Empirical absorption correction				
T_{min}	0.886	0.880	0.891	0.856
T_{max}	0.976	0.972	0.980	0.982
Refinement				
Data/parameters/restraints	6116/465/0	2828/249/0	4838/496/457	8346/564/180
Weighing factor	$w = 1/[2(Fo^2) + (0.0698P)^2 + 0.7596P]$	$w = 1/[2(Fo^2) + (0.0665P)^2 + 1.2752P]$	$w = 1/[2(Fo^2) + (0.0755P)^2 + 0.3931P]$	$w = 1/[2(Fo^2) + (0.0978P)^2 + 0.1958P]$
	$P = (Fo^2 + 2Fc^2)/3$	$P = (Fo^2 + 2Fc^2)/3$	$P = (Fo^2 + 2Fc^2)/3$	$P = (Fo^2 + 2Fc^2)/3$
R indices (observed)	$R_1 = 0.0424$	$R_1 = 0.0368$	$R_1 = 0.0413$	$R_1 = 0.0492$
	$wR_2 = 0.1225$	$wR_2 = 0.1072$	$wR_2 = 0.1230$	$wR_2 = 0.1580$
R indices (all data)	$R_1 = 0.0488$	$R_1 = 0.0415$	$R_1 = 0.0463$	$R_1 = 0.0664$
	$wR_2 = 0.1267$	$wR_2 = 0.1104$	$wR_2 = 0.1263$	$wR_2 = 0.1705$
GOF	1.097	1.077	1.082	1.098
S	1.097	1.077	1.038	1.116

Table 1 continued

Structure	DMG P1	DMG P2	DMG–water (1:1) S1	DMG–cyclohexanone (1:1) S2
Δ/σ_{max}	0.002	0.000	0.001	0.001
Δ/σ_{mean}	0.000	0.000	0.000	0.000
Residual electron density (e/Å³)				
Peak	0.472	0.337	0.389	0.377
Hole	−0.243	−0.207	−0.224	−0.224
rms	0.047	0.043	0.046	0.045

Table 2 Selected geometric parameters from crystal forms of 6,6′-dimethoxygossypol (DMG)

	DMG P1	DMG P2	DMG–water (1:1) S1	DMG–cyclohexanone (1:1) S2
Torsion angles (°)				
C1–C2–C12–C11[a]	100.2(1)	100.8(1)	−92.1(2)	−88.4(2)
C5–C6–O4–C31	−104.5(2)	−114.4(1)	116.0(1)	102.40(8)
C15–C16–O8–C32	129.8(1)	na	99.2(2)	118.8(2)
C10–C5–C23–H23[b]	35(1)	32.7(9)	12(1)	168.6
C10–C5–C23–H33[c]	na	na	na	−26.4
C20–C15–C28–H28[b]	167(1)	na	−34	8(1)
C20–C15–C28–H38[c]	na	na	164.6	na
RMS deviation of atoms from least-squared best-fit naphthalene plane (Å)				
Ring 1, atoms C1 through C10	0.016	0.083	0.065	0.015
Ring 2 atoms C11 through C20	0.108	na	0.051	0.048
Inter-naphthalene plane angle[d] (°)	104.86(4)	87.13(2)	85.86(4)	84.30(4)

[a] C1–C2–C2′–C1′ for **P2**

[b] Sole or major component

[c] Minor component

[d] Based on the best-fit planes through the ten atoms of each naphthalene ring

and H28 atoms (or between the H4 and H23 atoms in **P2**). This interaction is alleviated to a degree by rotation of the isopropyl group such that the methyl groups do not evenly straddle the sides of the naphthalene ring.

Because of internal symmetry, both isopropyl groups of **P2** necessarily have the same orientation, which is the more common outward positioning of the methyl groups (Fig. 2). In contrast, **P1** has one of its isopropyl methyl groups oriented outward and away from the center of the molecule and the other group oriented inward and toward the center of the molecule. For the two solvated DMG structures, one of each pair of the isopropyl groups is oriented with the methyl groups pointed outward, while the other isopropyl group is disordered occupying both outward and inward positions (Figs. 2, 3). For **S1**, the refined occupancy of the major part, which has the preferred outward orientation of the methyl groups, is 83.8(4)%. For **S2**, the major part is positioned in the less favored inward

orientation and has a refined occupancy of 67.5(6)%. All the isopropyl groups with the outward orientation are rotated away from a bisecting orientation of the isopropyl methyl groups by between 8° and 35° (Table 2).

For the disordered cyclohexanone molecule of **S2**, the refinement yielded occupancies of 77.8(4) and 22.2(4)% for the two chair conformers. These rings have Cremer–Pople puckering parameters ($q = 0.524$ Å, $\theta = 9.4°$, $\phi = 161°$ for the major part; $q = 0.527$ Å, $\theta = 6.3°$, $\phi = 136°$ for the minor part) that are close to those of an ideal chair (i.e., $\theta = 0°$). The puckering parameters of both rings are similar to the puckering parameters for crystalline cyclohexanone ($q = 0.536$ Å, $\theta = 8.2°$, $\phi = 170°$) [19].

Crystal packing

A number of packing motifs are observed within gossypol crystals [6]. One of the most commonly found associations

Fig. 3 Disorder within
6,6′-dimethoxygossypol (DMG)
solvates. a Isopropyl group
of DMG–water (1:1) (S1),
b isopropyl group of
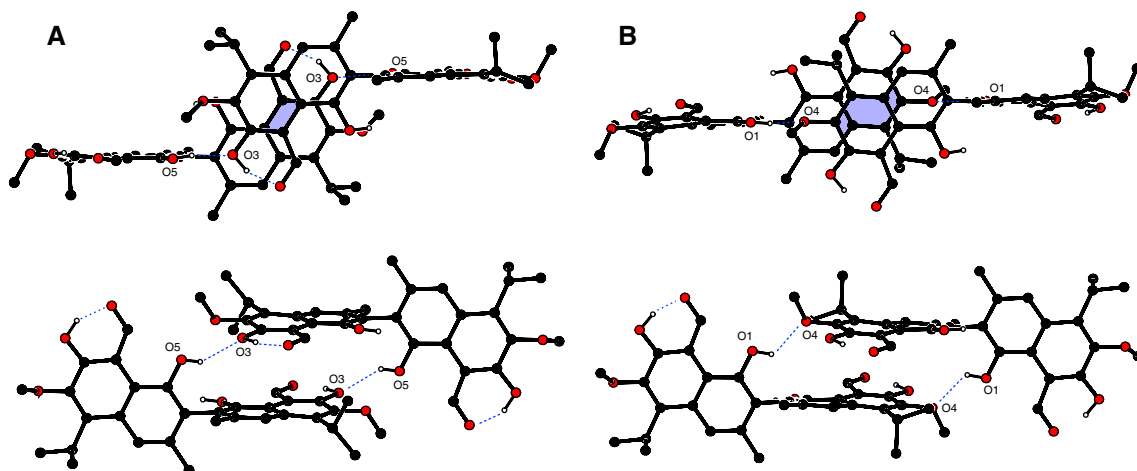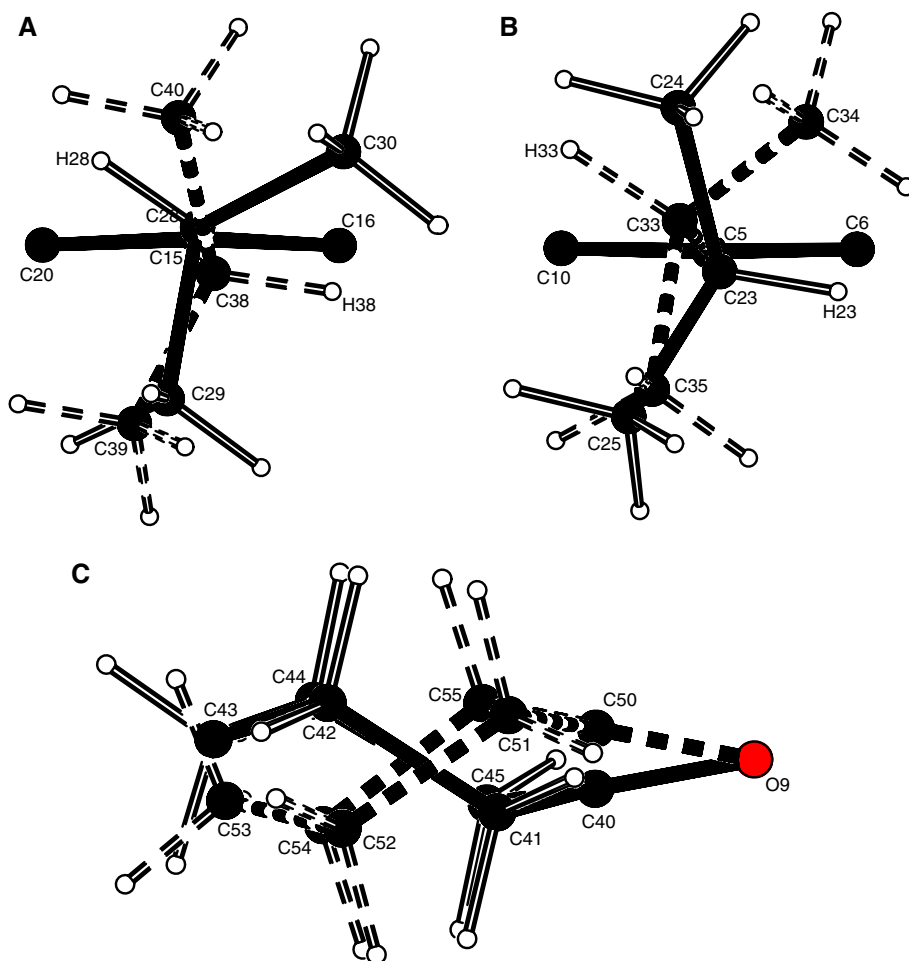DMG–cyclohexanone (1:1)
(S2), c cyclohexanone
molecule of S2

Fig. 4 Centrosymmetric dimers formed within 6,6′-dimethoxygossypol (DMG) crystals. a Top and side views of the assembly formed by the P1 form of DMG, b Top and side views of the assembly formed by the P2 form of DMG

is a centrosymmetric dimer that is stabilized by O5–H···O3 and O4–H···O5 hydrogen bonds and hydrophobic stacking between two of the naphthalene rings [6]. Despite the loss of the O4–H···O5 interaction in DMG because of methylation of the O4 hydroxyl group, this basic type of

centrosymmetric association is found in all the DMG structures reported to date. P1 (Fig. 4a) and S1 and S2 (not shown) have similar inter-molecular O5–H···O3 hydrogen bonds (Table 3). P2, in contrast, has a centrosymmetric dimer association that is shifted, such that its O1–H

Table 3 Hydrogen bonding in 6,6′-dimethoxygossypol (DMG) crystal forms

Structure/bonds	Symmetry	D–H, Å	H⋯A, Å	D⋯A, Å	D–H⋯A, °
DMG (**P1**)					
O1–H1⋯O6	x, 1½ − y, ½ + z	0.89(2)	2.06(2)	2.807(2)	140(2)
O3–H3⋯O2		0.87(2)	1.66(2)	2.480(2)	155(2)
O5–H5⋯O3	1 − x, 1 − y, 1 − z	0.82(2)	2.21(2)	2.947(1)	150(2)
O7–H7⋯O6		0.95(2)	1.62(2)	2.499(2)	152(2)
DMG (**P2**)					
O1–H1⋯O4	x, −y, ½ + z	0.86(2)	2.17(2)	2.778(1)	127(2)
O3–H3⋯O2		0.92(2)	1.62(2)	2.491(1)	157(2)
DMG–water (1:1) (**S1**)					
O1–H1⋯O9		0.85(2)	1.96(2)	2.727(2)	149(2)
O3–H3⋯O2		0.91(3)	1.61(3)	2.460(2)	155(2)
O5–H5⋯O3	−x, 2 − y, 2 − z	0.83(2)	2.14(2)	2.837(1)	142(2)
O7–H7⋯O6		0.92(3)	1.71(3)	2.524(2)	146(2)
O7–H7⋯O6	−1 − x, 3 − y, 1 − z	0.92(3)	2.53(3)	3.075(2)	118(2)
O9–H9A⋯O6	1 + x, y, z	1.00(3)	2.01(4)	3.013(2)	174(2)
O9–H9B⋯O7	−x, 3 − y, 1 − z	1.00(3)	2.37(3)	2.922(2)	114(2)
DMG–cyclohexanone (1:1) (**S2**)					
O1–H1⋯O9		0.84(3)	1.98(3)	2.748(2)	153(2)
O3–H3⋯O2		0.98(3)	1.52(3)	2.444(2)	155(2)
O5–H5⋯O3	−x, −y, 1 − z	0.91(2)	1.98(2)	2.724(2)	139(2)
O7–H7⋯O6		0.94(3)	1.57(3)	2.474(2)	158(3)

hydroxyl hydrogen atom (note the atom labeling differences for this compound) donates to the O4 methoxy oxygen atom (Fig. 4b) instead of the O3 hydroxyl oxygen atom. Correspondingly, there is greater overlap between the stacked naphthalene rings pairs and the extended planes of the non-stacked naphthalene rings are much less offset than is found in the more common DMG dimer association.

Packing of the centrosymmetric dimers in **S2** is similar to the packing of the gossypol molecules in the gossypol–cyclohexanone (1:1) crystal form [6]. However, in the gossypol complexes, the centrosymmetric assemblies form columns that are supported by O4–H⋯O8 intermolecular hydrogen bonds that are absent in the DMG solvate [6]. As in the gossypol–cyclohexanone (1:1) complex, the cyclohexanone molecules are tied to the DMG molecules through a hydrogen bond interaction between the O1 hydroxyl group and the carbonyl oxygen atom of the solvent (Table 3).

The DMG molecules of **S1** pack in an arrangement that is similar to the arrangement of the DMG molecules in DMG–acetic acid (1:1) [15] and similar to the packing of gossypol molecules in the Gdaniec et al. [6] Type-II gossypol solvates. Guest water molecules of **S1** are tied to the DMG dimers by an O1–H⋯O9 hydrogen bond, but also participate in two additional hydrogen bond interactions donating to the O7 hydroxyl oxygen atom and the O6 carbonyl oxygen atom of separate DMG molecules. The

former interaction acts as a bridge that ties adjacent DMG dimers into infinite columns (Fig. 5). The latter interaction helps tie adjacent columns together to form layers (Fig. 6). The layers have their polar groups all pointed inward, i.e., the layers have hydrophobic surfaces.

P1 packs in an arrangement that is similar to the known P1 polymorph of gossypol [6]. Centrosymmetric dimers are formed as above, but these do not assemble into extended column-like structures. Instead, a hydrogen bond is formed between the O1 hydroxyl hydrogen atom and the O6 carbonyl oxygen atom of a separate centrosymmetric dimer pair that extends in a direction essentially perpendicular to the long axis of the first dimer unit containing the O1 hydroxyl group. Pairs of these interactions tie the dimer assemblies into serpentine chains (Fig. 7), which are further stabilized by additional hydrophobic stacking between the naphthalene rings that are not part of the O5–H⋯O3-linked centrosymmetric dimers.

The packing motif of **P2** has not been previously observed among gossypol complexes; although, columns are formed that are similar in structure to those of Gdaniec et al. [6] Type-Va gossypol clathrates. Pairs of enantiomeric DMG molecules form centrosymmetric dimer units. However, each DMG molecule also forms a similar centrosymmetric unit with a second neighboring DMG molecule, resulting in a symmetrical column structure (Fig. 8). The difference in this polymorph's structure and the

Fig. 5 Column assemblies formed within the 6,6′-dimethoxygossypol (DMG) water clathrate (**S1**). Columns of DMG molecules are supported by hydrogen-bonded water molecules that bridge adjacent centrosymmetric dimer units
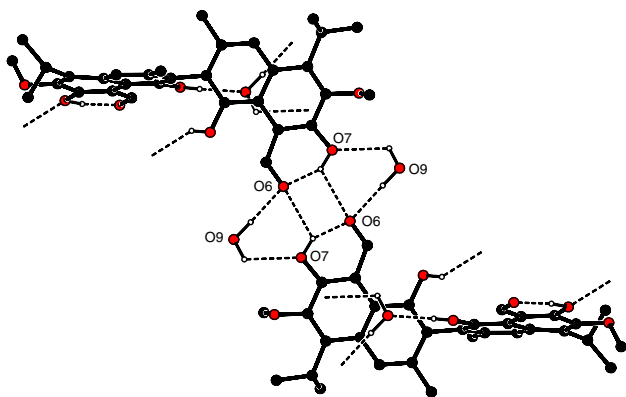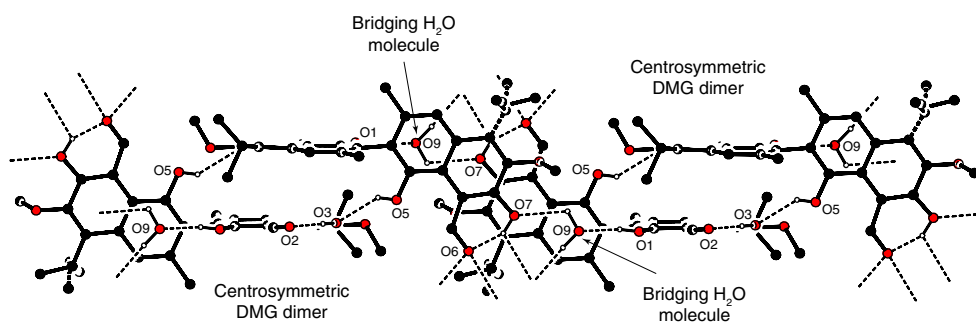

Fig. 6 Hydrogen bonding between neighboring 6,6′-dimethoxygossypol (DMG) dimer columns in DMG–water (1:1) (**S1**)

gossypol Type-Va structures [6] is the shift in the hydrogen bonding and greater overlap between the naphthalene ring pairs of the centrosymmetric dimer, which likely occurs to allow closer packing of the DMG columns. Gossypol Type-5 structures have been found with different gossypol-to-solvent ratios and showing variations in hydrogen bonding patterns [6], indicating that, among gossypol solvates of this type, some flexibility exists within the packing motif. One important difference is clear, however, forced

de-solvation of the Type-V gossypol structures results in destruction of the crystal form [6], while the packing arrangement with DMG is stable without any guest molecule.

Discussion

Gossypol forms solvates with many types of small organic compounds, including acids, alcohols, ketones, esters, ethers, benzyl, and other ringed compounds, as well as chlorinated and brominated compounds. Several aspects of gossypol's structure enable the compound to form a "host" matrix that can occlude many types of small molecules. The molecule has planer naphthalene rings that are interconnected by a bridge bond that has an approximate perpendicular orientation ($\sim$70–110°), which does not allow it to pack tightly by itself. In addition, the presence of several hydrogen bond acceptor and donor groups enables the molecule to form different intermolecular hydrogen bond arrangements. Finally, positioning of its hydrophilic pendent polar groups all on one side of the naphthalene ring and its hydrophobic pendent groups all on the other side of the ring allows the compound to pack such that it

Fig. 7 Association of centrosymmetric 6,6′-dimethoxygossypol (DMG) dimers within the DMG (**P1**) crystal form
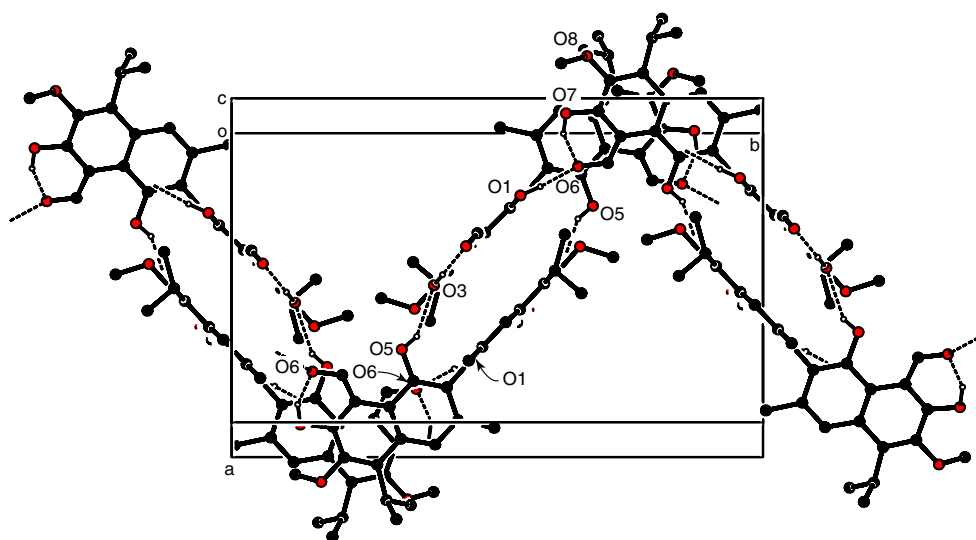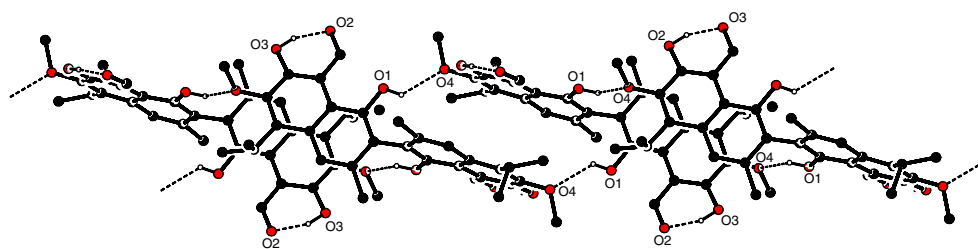
Fig. 8 Columns formed by overlapping 6,6′-dimethoxygossypol (DMG) centrosymmetric dimer units within the DMG (**P2**) crystal form

can form cavities and layers with varying degrees of hydrophobicity or hydrophilicity. These features all contribute to allow different associations of gossypol molecules to accommodate different types of guest molecules. As a result, several gossypol packing motifs are observed among gossypol solid-state structures. Gdaniec et al. [6] has classified these packing arrangements into 12 main types with several subtypes.

Methylation of the two 6-position hydroxyl groups of gossypol to form DMG reduces the number of hydroxyl groups that are capable of donating into intermolecular interactions. In addition, the methoxy groups are positioned such that they do not lie within the extended naphthalene planes; hence, they may allow for better packing of the DMG molecules without solvent. Therefore, one would expect that DMG would be less capable of forming different packing motifs in comparison with gossypol and that fewer solvates would be possible. With our current data, this seems to be observed as DMG does not form solvates with acetone, diethyl ether, chloroform, or pentan-2-one at conditions that gossypol readily form solvates with these molecules. On the other hand, DMG still has many of the same structural features of gossypol, including the perpendicular orientation of the naphthalene rings, the largely planer form, and the positioning of the pendent groups that allow DMG to retain the side-to-side polarity of the naphthalene rings. These features suggest that DMG should retain some of gossypol's ability to form solvates. The solvates formed between DMG and water, acetic acid, and cyclohexanone confirm this expectation.

It is curious that gossypol forms a Gdaniec et al. [6] Type-I packing arrangement with both acetone and cyclohexanone, but DMG forms this arrangement only with cyclohexanone. Although all the gossypol structures with this packing pattern have the O8–H hydroxyl hydrogen atoms donating to the O4 gossypol oxygen atom, there is considerable variation in the geometries of this intermolecular interaction within the Type-I complexes. This interaction appears to be relatively weak in gossypol–cyclohexanone (1:1), which has a long donor–acceptor oxygen distance of 3.75 Å. This weaker interaction occurs in order for the host lattice to accommodate the relatively large cyclohexanone molecule. Because DMG and cyclohexanone form a similar structure with the donating

hydroxyl group methylated indicates that this hydrogen bond is not critical to the packing arrangement. However, because DMG does not form the same arrangement with acetone, one cannot assume that DMG will form the same packing arrangement with other molecules known to form this arrangement with gossypol. This supports the proposal that DMG is more likely than gossypol to crystallize into non-solvated forms thereby making it even less likely to form solvates.

The DMG solvates formed with acetic acid and water pack in an arrangement that is similar to the Gdaniec et al. [6] Type-II gossypol solvates. Hence, methylation of the gossypol O4 and O8 oxygen atoms does not appear to prohibit the formation of this packing type. In the Type-II gossypol complexes, the O4 and O8 hydroxyl groups interact not with other gossypol molecules but with the guest molecule, which generally participate in several hydrogen bonds acting as both hydrogen donor and acceptor. The guest molecule effectively acts as a bridge molecule that ties centrosymmetric gossypol dimers into columns and layers. The formation of multiple DMG structures with this arrangement indicates that the loss of these guest-to-host interactions is not detrimental to the packing arrangement. Hence, DMG might be capable of forming solvates with other molecules known to form this packing arrangement with gossypol.

DMG crystallized from pentan-2-one did not result in a solvate, as occurs for gossypol crystallized from pentan-2-one [22]. Gdaniec et al. [6] refer to the packing arrangement between gossypol and pentan-2-one as Type X, a group of solvates that include many esters and ketones with six-to-eight carbon and oxygen atoms. In this packing motif, the gossypol molecules do not form centrosymmetric dimers, but instead form an association of the same enantiomer that is related by a two-fold rotation. The guest molecule resides within a symmetrical cavity formed between pairs of naphthalene rings that have both O1–H hydroxyl groups positioned on one wall of the cavity. These hydroxyl groups donate to the guest molecule's carbonyl oxygen atoms to form a di-gossypol-guest molecule sub-assembly. These sub-assemblies form chiral layers that are supported by O4–H and O8–H intermolecular hydrogen bonds, and there are no other intermolecular interactions. Methylation of the two hydroxyl groups

eliminates all the hydrogen bonding between the sub-assemblies, which likely destabilizes the packing arrangement. The formation of alternative crystal forms (e.g., a non-solvated polymorph or a water solvate) from DMG in solutions of mostly pentan-2-one supports this assessment. Hence, Type-X solvates are not likely to be formed between DMG and any of the other molecules known to form this type of packing arrangement with gossypol.

Conclusion

DMG, like gossypol, can exist in multiple solid-state forms. Two non-solvated polymorphs and three solvates of the compound are now known. Although there is less hydrogen bonding possible in DMG, four of the known structures pack in arrangements that are similar to related gossypol structures. The DMG molecules of **S1**, for which no equivalent gossypol clathrate has been reported, pack in an arrangement that is similar to DMG–acetic acid (1:1) [15] and the Gdaniec et al. [6] Type-II gossypol solvates. **P2** appears to pack in a highly ordered manner that has not been reported previously for gossypol, but is similar to the layers of gossypol molecules formed by the Gdaniec et al. [6] Type-Va structures.

Although DMG will form solvates, DMG does not appear to be as versatile of a host compound as gossypol because under conditions that gossypol readily crystallizes to form solvates, e.g., in acetone or diethyl ether at room temperature, DMG tends to form non-solvated polymorphs. Because we cannot rule out the possibility that DMG forms other complexes and because polymorphic forms of DMG exist, care is needed in the preparation of this compound to be sure that one understands exactly which form has been produced. Like gossypol, the physical properties (e.g., melting points, solubilities, etc.) and the bioactivity of DMG will likely vary depending on the exact solid-state form produced.

Supplemental material

The supplementary crystallographic data for this paper are contained in CCDC-743859 through CCDC-743862. These data can be obtained free of charge at www.ccdc.cam. ac.uk/conts/retrieving.html [or they can be obtained from the Cambridge Crystallographic Data Centre (CCDC), 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44(0)1223-336033; email: deposit@ccdc.cam.ac.uk].

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